

WHAT IS CLAIMED:

1. An optical device for receiving light, the device comprising:
a polarizing beam splitter (PBS) that substantially reflects light of a first
5 polarization and substantially transmits light of a second polarization orthogonal to the
first polarization;
a reflector positioned to reflect light transmitted by the PBS towards the PBS;
and
a detector positioned to detect light reflected by the PBS and/or light reflected
10 by the reflector.
2. The optical device of claim 1 wherein the reflector is angled relative to the
PBS.
- 15 3. An optical device for receiving light, the device comprising:
a polarizing beam splitter (PBS) which substantially reflects light of a first
polarization and substantially transmits light of a second polarization orthogonal to the
first polarization;
a reflector positioned to reflect light transmitted by the PBS towards the PBS,
20 wherein the reflector is angled relative to the PBS such that a light beam that has a non-
zero angle of incidence at the PBS is separated into a first beam having substantially
the first polarization and a second beam having substantially the second polarization;
and
a detector positioned to detect in the first or second beam.
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4. The optical device of claim 3, wherein upon reflection of the second beam
by the reflector, the angle between the first and second beam is at least 1°. (e.g.,
between 5 and 1°).
- 30 5. The optical device of claim 1 or 3, wherein the device is configured to
receive light having a wavelength between 380 nm and 780 nm.

6. The optical device of claim 5, wherein the device is configured to receive light having a wavelength between 400 nm and 680 nm.

7. The optical device of claim 1 or 3, wherein the PBS comprises substrate
5 having a first and second surface, at least one of which being coated with a substantially parallel array of elongated conducting elements, and the coated surface substantially reflects light of the first polarization and substantially transmits light of the second polarization.

10 8. The optical device of claim 3, wherein the detector comprises a first and second region, the detector being positioned to receive the first beam in the first region and the second beam in the second region.

15 9. The optical device of claim 1 or 3 wherein the reflector comprises a coating on the second surface of the PBS substrate, and the PBS second surface and the PBS first surface are angled.

20 10. The optical device of claim 3 further comprising a polarizer positioned in the path of the first beam, but not the second beam, wherein the polarizer is oriented substantially transmit light of the first polarization.

11. The optical device of claim 6 further comprising an optical element that directs light from the sample to the PBS.

25 12. A method of detecting fluorescence polarization of a sample, the method comprising:

exciting the sample with excitation light;

directing emitted light from the sample at the optical device of claim 3;

and

30 detecting light at the detector.

13. The method of claim 12 wherein the PBS comprises substrate having a first and second surface, at least one of which being coated with a substantially parallel

array of elongated conducting elements, and the coated surface substantially reflects light of the first polarization and substantially transmits light of the second polarization.

14. The method of claim 12 wherein the excitation light is polarized in a single
5 plane.

15. The method of claim 12 wherein the excitation light is circularly polarized.

16. The method of claim 8 wherein the detecting comprises detecting light in
10 the first and second beam.

17. The method of claim 16 wherein the light in the first and second beam are detected concurrently.

18. The method of claim 12 wherein the sample comprises a plurality of
15 regions.

19. The method of claim 18 wherein the detecting comprises concurrently
20 detecting light in the first and second beam for each region of the plurality of regions.

20. The method of claim 19 further comprising determining an FP value for each region of the plurality, the FP value being a function of the first polarity light and the opposite polarity light.

21. The method of claim 12 wherein the sample comprises a fluorescent
25 compound.

22. The method of claim 21 further comprising determining a parameter
30 descriptive of the fluorescence polarization of the fluorescent compound.

23. A method of detecting fluorescence polarization of a sample, the method comprising:

- exciting the sample with first polarized excitation light;
directing first emitted light from the sample at the optical device of
claim 3;
detecting light in the first and second beam to evaluate orthogonal
5 components of the first emitted light;
exciting the sample with second polarized excitation light, non-parallel
to the first polarized excitation light;
directing second emitted light from the sample at the optical device;
detecting light in the first and second beam to evaluate orthogonal
10 components of the second emitted light; and
determining a first value that is a function of the components of the first
emitted light and a second value that is a function of the components of the second
emitted light.
- 15 24. The method of claim 23, further comprising evaluating a function that
depends on the first and second values.
- 20 25. The method of claim 23 wherein the first and second polarized excitation
light have the same peak wavelength.
26. The method of claim 23 wherein the first and second polarized excitation
light have peak wavelengths that differ by at least 20 nm.
- 25 27. The method of claim 26 wherein the sample comprises a plurality of
fluorophores, each having a different spectral profile.
- 30 28. A polarizing beam splitter comprising:
an optically transparent substrate, having a front surface and a rear surface
angled relative to the front surface;
a generally parallel array of elongated elements disposed on the front surface of
the substrate configured to substantially reflect light of a first polarization, and
substantially transmit light of a second polarization orthogonal to the first polarization;
and

a reflective coating disposed on the rear surface.

29. The PBS of claim 28 wherein the elements are composed of aluminum or silver.

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30. The PBS of claim 28, wherein the array is configured to polarize light having a wavelength between 380 nm and 780 nm.

31. The PBS of claim 30 wherein the array is configured to polarize light
10 having a wavelength between 420 and 600 nm.

32. The PBS of claim 28 wherein the angle between the front and rear surfaces is between 5 and 50°.

15 33. The PBS of claim 28 wherein the reflective coating does not substantially alter the polarization of light that it reflects.

34. The PBS of claim 28 wherein the reflective coating is substantially uniform.

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35. The PBS of claim 28 wherein the substrate is a wedge.

36. The PBS of claim 28, wherein the rear surface is angled relative to the front surface such that a light beam that has a non-zero angle of incidence at the front surface
25 is separated into a first beam having substantially the first polarization and a second beam having substantially the second polarization, and upon reflection of the second beam by the reflector, the angle between the first and second beam is at least 1°.

37. A method of evaluating fluorescence polarization, the method comprising:
30 collecting light from a plurality of distinguishable positions on an illuminated object;

separating the collected light according to polarity using an optical element that reflects light polarized in a first plane and transmits light in a second plane, orthogonal to the first plane;

projecting the reflected light and the transmitted light onto a detector
5 surface; and

comparing the reflected and the transmitted light for each of the distinguishable positions to thereby determine fluorescence polarization at each of the distinguishable positions.

10 38. The method of claim 37 wherein the light in the second plane is reflected off a reflector towards the optical element.

39. A method comprising:

providing a plurality of spatially distinct nucleic acid samples and
15 amplification reagents that comprises a fluorophore attached to a nucleic acid primer;
concurrently amplifying each sample of the plurality; and
during the amplifying, concurrently detecting fluorescence polarization information associated with the fluorophore from each sample of the plurality, wherein the detecting comprises separating first and second polarity light using an element that
20 reflects first polarity light and transmits second polarity light, wherein the first polarity light is polarized in a first plane and the second polarity light is polarized in a plane orthogonal to the first plane.

40. The method of claim 39 wherein the first and second polarity light are
25 detected concurrently.

41. The method of claim 39 wherein the first and second polarity light are detected by the same detector.

30 42. The method of claim 39 wherein the element comprises an optically transparent substrate having a first and second surface and a parallel array of conductive material coated on the first surface.

43. An apparatus comprising:

the optical device of claim 3;

a light source;

5 a retainer configured to position a sample to receive light from the light source and to direct light emitted from the sample to the optical device.

44. The apparatus of claim 43 further comprising a thermal controller for regulating the temperature of the sample.

10 45. The apparatus of claim 43 further comprising the sample.

46. The apparatus of claim 43 wherein the retainer is configured to position a plurality of discrete samples to receive light from the light source and to direct light emitted from the sample to the optical device.

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47. The apparatus of claim 43 wherein the retainer is configured to position a multi-well container, a microscope slide, or an array.

48. The apparatus of claim 45 wherein the sample comprises a plurality of
20 spatially separated nucleic acid samples.

49. The apparatus of claim 44 wherein the thermal controller is configured to cyclically regulate temperature to effect a cycles that includes two or more of: nucleic acid annealing, extension, and denaturation.

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